Br. J. Pharmac. Chemother. (1967), **30**, 302–306.

ACTION OF SUBSTANCE P ON THE CENTRAL NERVOUS SYSTEM OF A GOAT

BY

C. A. BAILE AND H. MEINARDI*

From Harvard University, School of Public Health, Department of Nutrition, Boston, Massachusetts, and the Laboratories of the Rockefeller University, New York, New York, U.S.A.

(*Received November* 29, 1966)

In recent years a number of authors (Zetler, 1961 ; Cleugh, Gaddum, Mitchell, Smith & Whittaker, 1964 ; Meinardi & Craig, 1966) have been able to demonstrate the existence of several pharmacologically closely related principles, each of which would merit the designation of " Substance P " according to the original definition (Gaddum & Schild, 1934). Meinardi & Craig (1966) suggested that " Substance P "-like principles should be identified according to their laboratory of origin and to the species from which the principle has been isolated. (For example RUSP^g = " Substance P " isolated at the Rockefeller University from goat brains.)

Until recently it has remained doubtful whether or not substance P exists as such in the tissues or whether it is formed by the vigorous conditions used in the initial extraction process. These have usually involved boiling temperatures at pH 4 for a short time. In a previous report (Meinardi & Craig, 1966) it was shown that at least two, and possibly three, substance P-like active principles can be isolated from goat hypothalami by a very mild extraction procedure. One of these principles, RUSP^g, resembles closely, both chemically and pharmacologically, the highly purified substance P described by Vogler, Haefely, Hürlimann, Studer, Lergier, Strässle & Berneis (1963). RUSP^g appears to be a simple peptide of approximately 3000 M.W.

We wish to report on the effect of perfusing the brain ventricles of an unanaesthetized goat with an extremely low concentration of RUSP^g.

This appears to be the first time in 35 yr, in a variety of studies, that the action of substance P has been investigated on the same organ of the same species from which it had been originally extracted. The results indicate a definite and striking effect on the electrical activity recorded from the hypothalamus.

METHODS

Substance P was isolated from goat " hypothalami " by the method of Meinardi & Craig (1966).

The ventriculocisternal system of the brain of an unanaesthetized 1-yr-old male goat was perfused with " synthetic " cerebrospinal fluid (CSF) according to the method of Pappenheimer, Heisey, Jordan & deC Downer (1962). The rate of perfusion was 0.9 ml./min throughout, at a CSF pressure of 0 cm H_2O at the point of entrance in the skull. At intervals RUSP^g was added to the perfusion

* Present address: Epilepsy Centre, " Meer en Bosch," Heemstede, The Netherlands.

fluid. The establishment of a steady state was proven by periodic substance P bioassays (isolated guinea-pig ileum method) of the inflowing and outflowing solutions. Outflowing CSF was collected at 10 min intervals. When substance P was added to the perfusion fluid the third and fourth collection flasks contained equal concentrations of substance P as assessed by bioassay. This final concentration was slightly below the concentration of substance P in the inflowing solution.

On washing, more than half of the substance P had disappeared from the CSF collected in the second bottle. Detection of the lower limit was hindered by the fact that the ionic composition of CSF interferes with the activity of guinea-pig ileum.

The following data were obtained: CSF pressure, blood-pressure (through an intracarotid cannula), electrocardiogram, respiratory rate, body temperature and an electroencephalogram recorded over two electrodes, placed bilaterally in the anterior hypothalamus (in order to avoid confusion with a surface electroencephalogram we shall call this an electrohypothalamogram). During the course of the experiment we recorded data on an Electronics for Medicine polygraph for a period of at least 10 sec every 5 min. Blood samples were taken from a venous catheter for determination of blood glucose (auto-analyser) and insulin (Soeldner & Slone, 1965).

Experimental procedure. Two dose levels were investigated: synthetic CSF containing 4 p-mole RUSP^g/ml. (based on an M.W. of approximately 3000, in Euler Units 0.9 EU/ml.) and synthetic CSF containing 40 p-mole RUSP^g/ml.

The specificity of the observed effects was partially tested by incubation of RUSP^g with chymotrypsin and subsequent denaturation of the chymotrypsin by boiling the mixture. An aliquot of this mixture was taken such that a dose in weight (not activity) equivalent to 40 p-mole/ml. intact RUSP^g resulted.

The following sequence was used: 85 min synthetic CSF; 35 min at 4 p-mole RUSP^g/ml; 60 min synthetic CSF; 35 min at 40 p-mole RUSP^g/ml; 50 min synthetic CSF; 35 min at 40 p-mole RUSP^g/ml; 50 min synthetic CSF; perfusion interrupted for 4 hr, 10 min synthetic CSF; 35 min chymotrypsin degraded RUSP^g, originally 40 p-mole RUSP^g/ml; 50 min synthetic CSF; 35 min at 40 p-mole RUSP^g/ml; and 50 min synthetic CSF.

RESULTS

None of the recorded parameters changed at the dose level of 4 p-mole RUSP^g/ml., but at 40 p-mole RUSP^g/ml. all recorded parameters remained stable except the electrohypothalamogram. Selected representative sections of these recordings are produced in Fig. 1. Figure 2 shows the frequency in which the amplitude during each 10 sec record of the electrohypothalamogram exceeded 100 μV or 1000 μV. In the same figure, deviations of the systolic or diastolic blood pressure larger than 5 mm Hg are recorded. After 6 min of the higher substance P dose, a few bouts of high amplitude slow waves were noted. These became very numerous immediately following the 12th and the 23rd min of infusion. These bursts of high amplitude activity continued to recur for a considerable time after the perfusion with substance P had terminated; in fact, following the second administration of substance P at the 40 p-mole/ml. level, 3 hr were required before the electrohypothalamogram had reached apparent quiescence. One hour after return to base level activity, perfusion with chymotrypsin degraded substance P was initiated; no changes were observed. After another perfusion with the 40 p-mole/ml. level of active substance P it was found that the elicited bursts of activity were not as dramatic as before. Nonetheless, the electrohypothalamogram was definitely perturbed according to a pattern similar to that noticed during the initial perfusions with active substance P.

Behavioural changes were inconspicuous. The animal seemed more tranquil than usual during experiments of similar duration. It reacted instantaneously, however, with

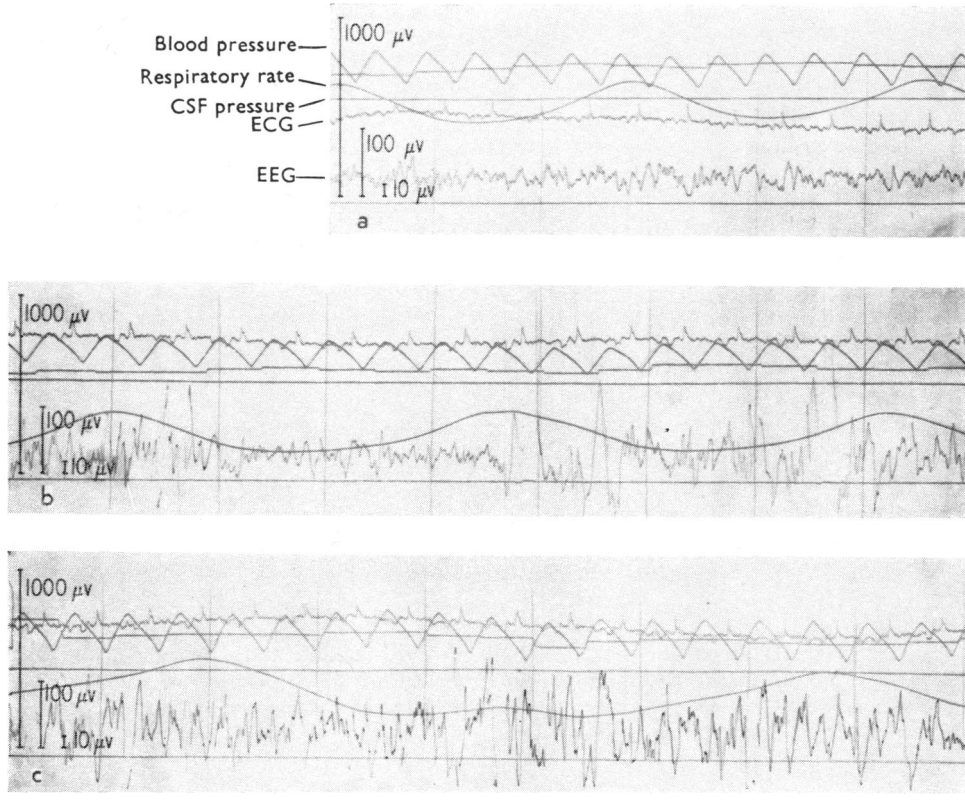

Fig. 1. Each vertical line represents 1 sec. a. Record before administration of RUSP^g (20 min after start of the experiment). b. and c. Arbitrary sections of record during perfusion with "CSF" containing 40 p-mole RUSP^g/ml. (b. 217 min after start of the experiment; c. 304 min after start of the experiment).

movements of head and ears when approached. The pupils appeared somewhat narrower than those of its fellow goats in identical conditions. Three times within the first 6 min of infusion of CSF containing active substance P, defaecation or movements of the hind quarters occurred; this may well have been coincidental. There were no significant changes in blood glucose or insulin levels associated with the different phases of the perfusions.

DISCUSSION

Although this is essentially a pilot experiment, we feel justified in reporting the results for the following reasons. First, this experiment clearly shows that an extremely small amount of highly purified peptide is capable of producing clear-cut and characteristic perturbations of the electrohypothalamogram. The route of administration caused a specific response and not a general response, as is shown by the absence of changes in blood pressure, body temperature, or motor phenomena; this strongly suggests a specific target. Whether other peptides will have similar effects in such low concentrations needs further investigation.

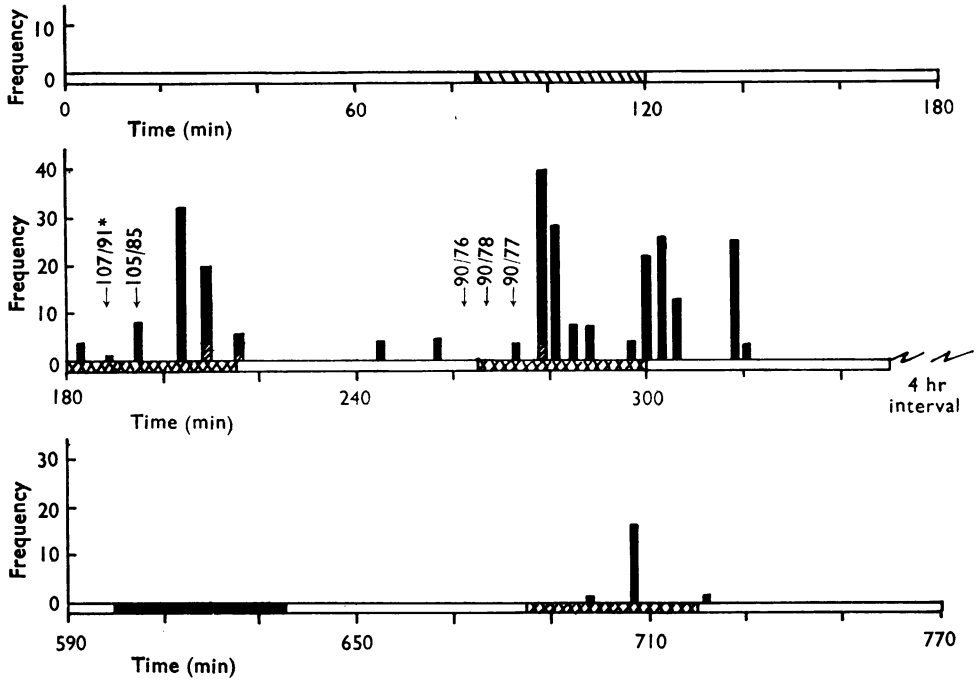

Fig. 2. Electrohypothalamogram and blood pressure (noted only when deviation of blood pressure from mean exceeded 5 mm Hg) data from perfusion of the ventriculocisternal system of a goat with the following solutions: ☐ "CSF" (synthetic cerebro-spinal fluid); ▨ "CSF"+4 p-mole Rockefeller University Substance P from goat brains (RUSP^g)/ml.; ▩ "CSF"+40 p-mole RUSP^g/ml; ■ "CSF"+40 p-mole chymotrypsin inactivated RUSP^g/ml. The vertical bars represent the frequency in which the amplitude during each 10 sec record of the electrohypothalamogram exceeded 100 uV (vertical black bar) or 1,000 uV (vertical hatched bar), *Blood pressure (mm Hg).

Secondly, the interest in substance P has suffered a rather sharp decline since it was reported (Boissonnas, Franz & Stürmer, 1963; Vogler *et al.*, 1963; Zuber, 1963) that substance P is extremely difficult to purify and that the purest preparations obtainable are devoid of any action on the central nervous system. It has been argued by Krivoy (Vogler *et al.*, 1963, p. 389) that the absence of activity on the central nervous system of the highly purified preparation might have been due to the fact that the isolation was monitored on the basis of musculotropic action. However, the purification of substance P according to the method of Meinardi & Craig (1966), monitored exclusively by guinea-pig ileum bioassay, yielded a preparation of high purity with demonstrable high neurotropic activity. Our positive results are in evident contrast to the negative findings of Haefely & Hürlimann (1962) and it is tempting to argue that this may be due to the fact that we have used substance P obtained from the same species and the same organ on which it was tested. Such an argument, however, may not be justified since Haefely & Hürlimann did not report on intracerebral electroencephalographic recording, nor did they use an equivalent route of administration. The primary site of action of the

perfused substance P cannot as yet be deduced from our experiment since only two electrodes were used.

SUMMARY

A naturally occurring highly purified polypeptide " Rockefeller University Substance P " obtained from goat hypothalami directly or indirectly influences the electrical activity of that area.

This work was supported in part by grants-in-aid from the National Institutes of Health (AM 02493-0 to Dr. Lyman C. Craig), N.I.H., U.S.P.H.S.; the National Institute of Neurological Diseases and Blindness (NB-01941 to Dr. Jean Mayer), N.I.H., U.S.P.H.S.; the National Science Foundation (GB-4594 to Dr. C. A. Baile and Dr. J. Mayer); and the Fund for Research and Teaching, Department of Nutrition, Harvard University School of Public Health.

REFERENCES

BOISSONNAS, R. A., FRANZ, J. & STÜRMER, E. (1963). On the chemical characterization of substance P. *Ann. N.Y. Acad. Sci.*, **104**, 376–377.

CLEUGH, J., GADDUM, J. H., MITCHELL, A. A., SMITH, M. W. & WHITTAKER, V. P. (1964). Substance P in brain extracts. *J. Physiol.*, **170**, 69–85.

GADDUM, J. H. & SCHILD, H. (1934). Depressor substances in extracts of intestine. *J. Physiol.*, **83**, 1–14.

HAEFELY, W. & HÜRLIMANN, A. (1962). Substance P, a highly active naturally occurring polypeptide. *Experientia*, **18**, 297–303.

MEINARDI, H. & CRAIG, L. C. (1966). Studies of Substance P. In *Hypotensive Peptides*, Proc. Intern. Symp., Florence, Italy, 1965. Ed. ERDÖS, E. G., BACK, N. & SICUTERI, F. Pp. 594–607. Springer-Verlag, New York.

PAPPENHEIMER, J. R., HEISEY, S. R., JORDAN, E. F. & DOWNER, J. de C. (1962). Perfusion of the cerebral ventricular system in unanesthetized goats. *Am. J. Physiol.*, **203**, 763–774.

SOELDNER, J. S. & SLONE, D. (1965). Critical variables in the radio-immunoassay of serum insulin using the double antibody technic. *Diabetes*, **14**, 771–779.

VOGLER, K., HAEFELY, W., HÜRLIMANN, A., STUDER, R. O., LERGIER, W., STRÄSSLE, R. & BERNEIS, K. H. (1963). A new purification procedure and biological properties of substance P. *Ann. N.Y. Acad. Sci.*, **104**, 378–390.

ZETLER, G. (1961). Zwei neue pharmakologisch aktive Polypeptide in einem Substanz P-haltigen Hirnextrakt. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **242**, 330–352.

ZUBER, H. (1963). Isolation of substance P from bovine brain. *Ann. N.Y. Acad. Sci.*, **104**, 391–392.